WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1482-1488. Research Article ISSN 2277– 7105

TREATMENT OF PHARMACEUTICAL EFFLUENT USING PHOTOSYNTHETIC PURPLE NONSULFUR BACTERIUM *RHODOPSEUDOMONAS ACIDOPHILA* OUPNSB. 1

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Article Received on 07 May 2015,

Revised on 28 May 2015,
Accepted on 19 June 2015

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ABSTRACT

A photosynthetic purple non-sulphur bacterium *Rhodopseudomonas acidophila* OU PNSB 1 isolated from waste water that has high growth and hydrogen production was used to treat pharmaceutical effluent in a 3L Bioreactor. There is a reduction of 74% of COD, 22% of BOD and 65% of Organic Matter with simultaneous hydrogen production. This may be due to degradative activity of the bacterium with a variety of enzyme systems. The pH drifted towards alkalinity due to the growth of bacterium. The data clearly demonstrates that the bacterium is effective in transforming complex waste water into useful products. The present study encourages the development of technology particularly for industrial waste treatment and simultaneous production of clean energy (hydrogen).

KEYWORDS: photosynthetic bacteria, waste water treatment, purple non-sulfur bacteria, Pharmaceutical effluent, Hydrogen.

INTRODUCTION

The growth of industries in this century has generated a wide variety of anthropogenic (man made) compounds. Such compounds can cause problems of disposal and, if they enter the environment, can have a detrimental effect. Pollution control is a rapidly expanding area of interest because it has become a major problem of higher magnitude and urgency. Recent environmental awareness and legislative restrictions on uncontrolled discharges of wastes have highlighted the need for new technologies for the treatment of wastes (Cokgor *et al*, 2004; Ren *et al*, 2008). Microbial treatment of wastes is a well-established technology and exploited efficiently with mixed wastes, organic wastes and other wastes (Bitton, 2005; Huseyn *et al*, 2006).

In nature Purple non sulfur bacteria occur in fresh water, brackish waters, marine habitats, oceanic water, tropical mangrove and salt marsh environment, waste waters and industrial effluents (Sasikala *et al*., 1995; Renuka, *et al* 1987; Kantachote *et al*, 2005; Uma Devi *et al*, 2008 and Madukasi *et al*, 2010, 2012). Utilization of anoxygenic phototrophic bacteria for treatment of wastes is beneficial over other methods: the system requires limited space, short period of time, simple technology, cost effective and ecologically inoffensive. Major dilution is not required hence can be used in places of water scarcity. Additional sludge disposal treatment is not created because the bacterial biomass is rich in proteins, vitamins and carotenoids (Bertling *et al*, 2006). The sludge treatment by photosynthetic bacteria requires neither autoclaving nor processing.

Phototrophic bacteria have the capability of utilizing many organic substrates as carbon sources/ electron donors hence are used to treat many wastes containing organic compounds. The photosynthetic bacteria are used for treatment of wastes for the removal of BOD, COD, TOC and degradation of toxic compounds. The main aim of this study is to test the ability of the isolated strain of purple non sulfur bacterium *Rhodopseudomonas acidophila* OU PNSB-1 and to develop economically viable system using wastewater as substrate with purple non-sulfur bacteria for treatment of wastewater and simultaneous hydrogen production.

MATERIALS AND METHODS

Bacterial culture

A purple non sulfur bacterium isolated from industrial waste water is used to treat Pharmaceutical effluent. The bacterium was isolated with a modified AT-medium (Imhoff, 1988) described for the isolation and cultivation of purple non sulfur bacteria.

Sample Collection

Waste water from a pharmaceutical company located at Balanagar, Hyderabad was collected at different times, and analysed for COD, BOD and Organic Carbon in the laboratory following standard procedures (APHA, 1992). The properties of wastewater varied at each collection time hence it was centrifuged for 10 minutes followed by autoclaving at 121º C for 15 min to achieve sterile condition prior to use as the growth medium.

Effluent Treatment

The effluent was filtered through Whatmann No 1 filter paper and diluted to different proportions with deionised water to obtain required dilutions. The pH initially adjusted to neutral and inoculated with bacterial suspension for treatment and hydrogen production.

Hydrogen production studies

The hydrogen produced was measured by the method described by Kumazawa and Mitsui (1981) and Vincenzini *et al*. (1982). The log phase cultures of photosynthetic bacteria were harvested by centrifugation at 10,000 rpm for 10 minutes, washed thrice with 0.3% saline and suspended in basal medium devoid of electron donor and nitrogen source.

5 ml of the bacterial suspension was taken in a 12 x 125 mm rimless test tube. The test tube was then sealed with suba seal and gas phase was replaced with 100% argon by repeatedly evacuating and flushing the tube. Hydrogen produced was measured by injecting 0.5 ml of the gas phase from the reaction vessels with a air tight syringe into a gas chromatograph (CIC make fitted with molecular sieve 5A column (2m x 1/8' OD SS column, 60-80 mesh) to a thermal conductivity detector (T.C.D). Gas analysis was done with the following parameters: Oven temperature 60^0C, Nitrogen as carrier gas (Flow rate 30 ml/min), 120 mA detector current. 0.5 ml of gas phase that is taken out was replaced every time with argon to maintain positive pressure. The amount of hydrogen was calculated from peak heights obtained using a calibration curve that is prepared using standard hydrogen.

RESULTS AND DISCUSSION

Treatment and utilization of pharmaceutical effluent by biological systems is very important from the view point of production of biomass as well as removal of pollutants at comparatively cheaper cost. Purple non-sulfur bacteria require an external electron donor for growth and biomass. Since they have the capacity to utilize a wide range of organic and inorganic compounds as electron donors, the use of pharmaceutical effluent containing unused organic and inorganic compounds might provide cheap raw materials for biomass. The effluents generally contain high concentration of nutrients which support the growth of bacteria. The various physico-chemical parameters of the effluent are given in the Table-1. It was observed that the pharmaceutical effluent was little alkaline with considerable organic matter and served as carbon/electron source for the growth of the selected bacterial strain OU PNSB-1.The growth of the purple non sulfur bacterium was studied in different concentrations of sterile effluent under anaerobic conditions (Table-2). Pharmaceutical effluent supported good growth of the strain under study and attained maximum growth between 6^{th} and 7^{th} day of incubation. The increasing growth of the strain *Rps. acidophila* OU.PNSB.1 with increasing concentration of wastewater to 80%, show that the effluent was not toxic to the growth of the organism. The pharmaceutical effluent at a concentration of 90% produced moderate amount of hydrogen at a rate of 4.8 ml/ mg dry wt/h. This was about 25% of the hydrogen produced with the use of synthetic substrates (data not shown).

Table. 1: Physico-chemical parameters of Pharmaceutical wastewaters.

S. No	Parameter	Concentration (ppm)
1	Appearance	Transparent
2	Colour	Pale yellow
3	pH	7.8
4	Total solids	1, 200
5	Suspended solids	190
6	Sulfates	1500 – 2000
7	Reducing sugars	385
8	Organic matter	35.2
9	COD	1022
10	BOD	511

Table. 2: Growth of *Rps. acidophila* OU. PNSB. 1 in Pharmaceutical wastewaters

Percentage of effluent	Growth in pharmaceutical effluent	µl of H2 produced
10	+	60
20	+	95
30	+	133
40	+	202
50	+	210
60	+	405
70	+	620
80	++*(2.32)	740
90	+	980
100	….	602

*+ = moderate; ++ = good; .... = poor; - = no growth * Bchl mg/100 ml*

Cultures grown anaerobically under light (3000 lux), Temperature 30^0C and pH 7.0

It was tested further for treatment and large scale hydrogen production using a 3L bioreactor. The effluent at 90% concentration was analyzed for BOD, COD and organic matter before and after treatment, and the values are given in table-3. The results reveal that there is a reduction of about 74% of COD, 22% of BOD and 65% of organic matter with simultaneous production of hydrogen at a rate of 4.0- 4.5ml/g drywt/h. Thus *Rps. acidophila* OU PNSB 1 not only evolved considerable amount of hydrogen but also useful for the treatment of wastewater to a large extent.

Table. 3 : Pharmaceutical effluent treatment and simultaneous hydrogen production by *Rps. acidophila*. OU. PNSB.1

Parameter	Before treatment (ppm)	After treatment (ppm)	Difference (% reduction)
BOD	460	360(78.2%)	100(21.8%)
COD	920	240 (26.1%)	680 (73.9%)
Organic matter (oxidisable)	31.68	11.16 (35.2%)	20.52 (64.8%)
*H_2 produced	-	4.0 – 4.5 ml	-

Results expressed after 72 h. Cutures grown anaerobically under light

Intensity of 4000 lux, temperature 30º C and pH 7.0

** Rate of hydrogen expressed h^{-1} g dry. wt^{-1}*

The development of wastewater treatment process by using purple non sulfur bacteria has created considerable interest (Livia et al, 2006; Madukasi et al, 2011, 2014; Ramchander merugu, 2014 and).The results revealed that the organic matter present in the pharmaceutical waste was assimilated by the strain OU PNSB 1.It is evident that COD 74% reduction after treatment can be comparable to observations of 80% COD reduction with pharmaceutical waste using *Rhodobacter spheroids* Z08 (Madukasi *et al*, 2010)and 88.7% reduction using *Rhodopseudomonas* (Vinay kumar and Venkat Ramana). The organic matter reduction (65%) is s comparable with the results of Mitsui *et al*.(1985) using orange processing waste water.

Further work on scale up of the process is required for adaptation to field conditions. An attempt was also made to study the growth without maintaining aseptic conditions since stabilization process is unwieldy and would not be feasible under aseptic conditions.

CONCLUSION

- The results obtained clearly demonstrate that the *Rhodopseudomonas acidophila* OU PNSB-1 is effective in transforming complex wastewater into useful products.
- The biomass obtained can also be harvested and made use as SCP or energy production. Further focus should be on the development of a long term process of waste treatment at a minimum cost and production of clean energy.

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